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Survival of Cowpea Rhizobia in Soil as Affected by Soil Temperature and Moisture

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Successful inoculation of peanuts and cowpeas depends on the survival of rhizobia in soils which fluctuate between wide temperature and moisture extremes. Survival of two cowpea rhizobial strains (TAL309 and 3281) and two peanut rhizobial strains (T-1 and 201) was measured in two soils under three moisture conditions (air-dry, moist (−0.33 bar), and saturated soil) and at two temperatures (25 and 35°C) when soil was not sterilized and at 40°C when soil was sterilized. Populations of rhizobia were measured periodically for 45 days. The results in nonsterilized soil indicated that strain 201 survived relatively well under all environmental conditions. The 35°C temperature in conjunction with the air-dry or saturated soil was the most detrimental to survival. At this temperature, the numbers of strains T-1, TAL309, and 3281 decreased about 2 logs in dry soil and 2.5 logs in saturated soil during 45 days of incubation. In sterilized soil, the populations of all strains in moist soil increased during the first 2 weeks, but decreased rapidly when incubated under dry conditions. The populations did not decline under saturated soil conditions. From these results it appears that rhizobial strains to be used for inoculant production should be screened under simulated field conditions for enhanced survival before their selection for commercial inoculant production.

The benefits from inoculation depend on survival of introduced rhizobia in the soil. They must survive long enough after sowing to nodulate the host, and it would be desirable for the rhizobia to persist between cropping seasons. Soil moisture content and temperature are factors that strongly influence survival of rhizobia. In a field environment soil moisture content and temperature are dynamic and fluctuate between wide ranges. Many countries have distinct wet and dry seasons; soil temperatures fluctuate in subtropical climates, but remain relatively high all year in tropical countries. Cowpeas and peanuts are economically important food legumes that are nodulated by rhizobia in the cowpea cross-inoculation group. These legumes are adapted to subtropical and tropical regions.

The selection of rhizobia for inoculants should include evaluations of their ability to survive in soil (2). Published data indicating the importance of soil moisture, temperature, and interactions between these factors on survival of cowpea rhizobia are very limited. *Rhizobium* as a genus is sensitive to low soil moisture conditions. Survival in soil maintained at low water tension (near field capacity) is much superior to survival under conditions of high water tension (3, 5, 8). Generally the population declines by 2 logs within a few days when a moist soil undergoes drying at moderate temperature (3, 8). Evidence indicating consistent differential sensitivity of fast- and slow-growing rhizobia is not conclusive (1, 3, 8). Strains of cowpea rhizobia differ in their susceptibility to desiccation in silica sand at 27°C (3). Populations of some strains declined by 1 log in 3 weeks, whereas the populations of other strains declined by 3 logs.

Data are not available on survival of cowpea rhizobia in soil maintained under moisture and temperature conditions frequently encountered in field environments. Because of the limited amount of information on survival of cowpea rhizobia and their importance in tropical and subtropical agriculture, experiments were undertaken in our laboratory to evaluate the effect of soil temperature, moisture, and their interaction on survival of cowpea rhizobia.

MATERIALS AND METHODS

Soils. The survival of *Rhizobium* in the cowpea cross-inoculation group was investigated by using two soils. A Korat sandy loam was obtained from Thailand by air freight and is a ustoxic dystopepts with a pH of 5.7 in a 2:1 (wt/wt) water-soil suspension. The other soil, a Lufkin sandy clay loam, was obtained in Texas and is a Vertic Albaqualf with a pH of 6.8. Both soils are well suited to cowpea production, but only the soil from Thailand is commonly used for peanut produc-

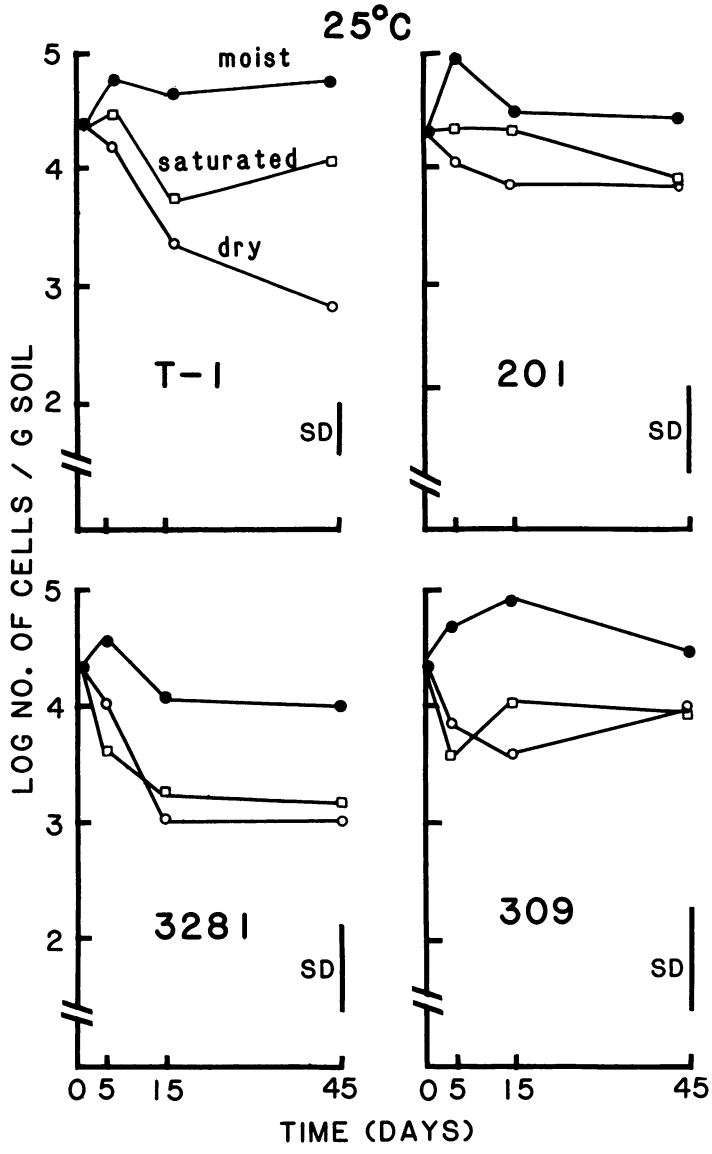

FIG. 1. Survival of cowpea rhizobia strains in nonsterilized dry, moist, and saturated soil incubated at 25°C.

tion. The particular areas from which soil was collected were not known to have been planted to cowpeas or peanuts. Siratro (*Macroptilium atropurpureum*) was grown on samples of soil and did not nodulate, indicating the absence of cowpea rhizobia.

Strains and inoculum. Two strains of *Rhizobium*, TAL309 and 3281, which nodulate cowpeas were utilized in the investigations and were respectively obtained from the NifTAL culture collection (Paia, Hawaii) and the U.S. Department of Agriculture culture collection (Beltsville, Md.). Two other strains, 201 and T-1, were respectively isolated from field-grown peanuts in Thailand and Texas. Inoculum was prepared by growing rhizobia in yeast extract-mannitol broth (10) to a cell density of approximately 4×10^8 cells per ml, which corresponded to the late log phase of growth.

Inoculation. Each strain of *Rhizobium* was inoculated into 10-g samples of air-dry soil contained in test tubes (15 by 100 mm) with plastic caps. Inoculation of sterilized soil was accomplished by adding 0.5 ml of broth culture to each tube. For nonsterilized soil a 1:10 dilution of broth was made with 0.1% peptone, and 0.5 ml was used as the inoculum. Moisture was distributed by shaking individual tubes by hand until the moisture appeared evenly distributed as was apparent by uniform soil color.

Moisture treatments. Distilled water was added after inoculation to prepare the moisture treatments: −0.33 bar moisture tension and flooded (1-cm water layer

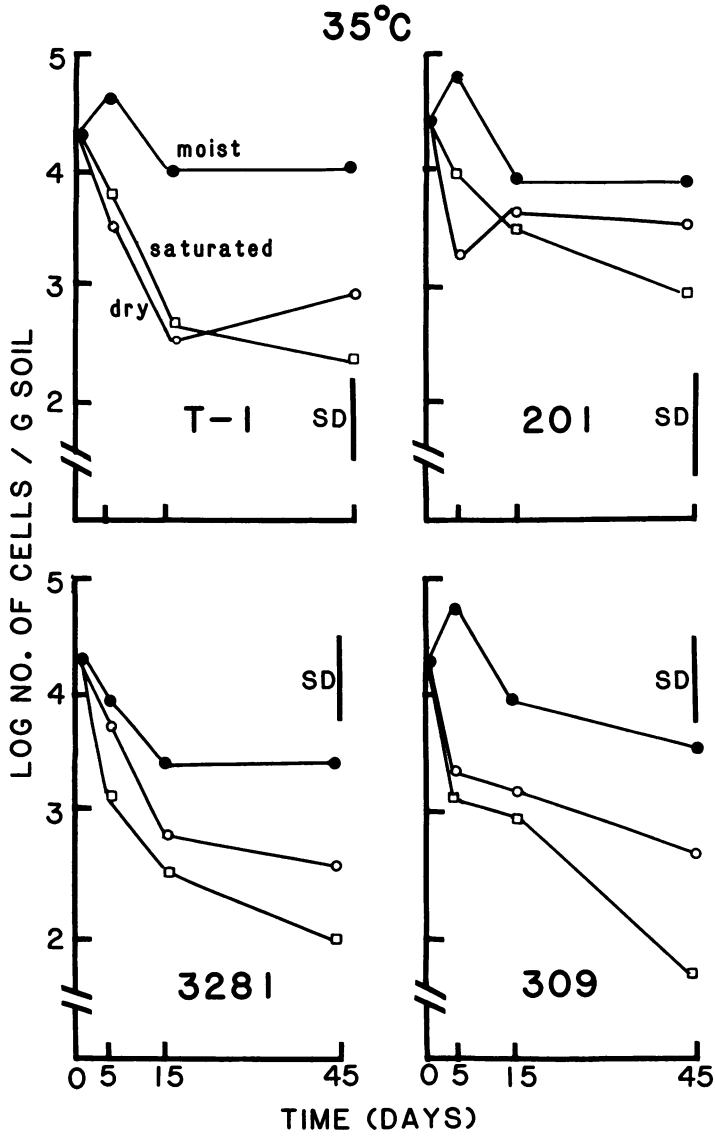

FIG. 2. Survival of cowpea rhizobia strains in nonsterilized dry, moist, and saturated soil incubated at 35°C.

over soil surface). A pressure membrane apparatus was used to determine soil moisture content at −0.33 bar moisture tension. The dry treatment was achieved by placing inoculated samples, with caps removed, in a laminar-flow hood for 2 h after which the soil appeared dry. The water content of moist samples was maintained during incubation by keeping them in a plastic humidity chamber lined with moist paper towels. Periodic weighing of samples indicated insignificant moisture loss. The samples for the dry treatment were exposed directly to the air in the incubator.

Soil sterilization. Soil was sterilized by autoclaving the 10-g samples in test tubes for 1 h on each of 2 successive days.

Enumeration of rhizobia. Rhizobia inoculated into nonsterilized soils were enumerated by the most probable number method (11). Siratro was the indicator plant and was examined for nodulation 3 weeks after inoculation. Four replications and 10-fold serial dilutions in plant nutrient solution were used in the most probable number procedure. Rhizobia inoculated into sterilized soil were enumerated by making 10-fold dilutions in 0.1% peptone and spread plating 0.1 ml on yeast extract-mannitol agar (10). Cultures were incubated for 10 days at 29°C before counting. Two soil samples of each treatment were processed at each sampling, and the entire 10-g samples were utilized.

RESULTS AND DISCUSSION

Survival of rhizobia in the two nonsterilized soils was not significantly different ($\alpha = 0.10$),

TABLE 1. Survival of cowpea rhizobial strains in sterilized soil incubated at different moistures at 40°C

Strain	Moisture	Survival[a] after incubation time (days):			
		0	5	15	45
201	Dry	7.53	6.53	6.25	6.41
	Moist	7.53	8.15	8.20	8.17
	Saturated	7.53	7.32	7.28	7.92
T-1	Dry	7.53	4.99	5.00	4.30
	Moist	7.53	8.15	8.20	8.17
	Saturated	7.53	7.32	7.28	7.92
TAL309	Dry	7.25	6.32	6.28	5.43
	Moist	7.25	7.30	7.66	7.36
	Saturated	7.25	6.80	6.83	6.84

[a] Data are presented as log units per gram of soil; standard deviation, ±0.32.

and there was no significant ($\alpha = 0.10$) interaction of soil types with other factors. For brevity, only averages for the two soils are presented.

Inspection of the data for rhizobial survival at 25°C (Fig. 1) reveals that strains 201 and TAL309 maintained relatively high populations for 45 days under all moisture conditions. Strain T-1 withstood saturated soil conditions better than dry soil conditions (Fig. 1). Survival of strain 3281 under saturated and dry soil conditions was similar and relatively poor as compared with survival under moist conditions (Fig. 1).

Incubation of rhizobia at 35°C was more detrimental than at 25°C, but again strain 201 survived well relative to the other strains (Fig. 2). For all strains the tendency was to have more viable rhizobia present at 45 days in soil maintained under dry conditions than under saturated conditions (Fig. 2). The standard deviation was too large for statistical significance between these moisture treatments for individual strains, but combined analysis of variance indicated that the saturated conditions were significantly ($\alpha = 0.05$) more detrimental than the dry conditions. The contrast in survival of strain TAL309 between the different moisture treatments and temperatures (Fig. 1 and 2) indicates the importance of strain evaluations being made for several conditions.

The results obtained with nonsterilized soils provided no opportunity of separating the effects of biotic and abiotic factors on rhizobial survival. A second inoculation experiment was conducted with a sterilized soil to eliminate biotic factors. Strain 3281 was not included to reduce labor requirements, and the incubation temperature was increased to 40°C to increase pressure on survival. Under moist soil conditions rhizobial populations did not decline for 45 days (Table 1). They also survived very well under saturated conditions (Table 1), in contrast to the results with nonsterilized soils (Fig. 1 and 2). The population actually increased for strains 201 and T-1 under saturated soil conditions and only slightly decreased for strain TAL309 (Table 1). Mahler and Wollum (6) also reported that after an initial reduction in the population of *Rhizobium japonicum* in sterilized soil the population increased. Apparently after a period of adjustment rhizobia can reproduce in soil when competitors are eliminated. Under dry moisture conditions the populations declined for all strains (Table 1), but as occurred for the nonsterilized soils (Fig. 1 and 2), strain 201 indicated better survival than the other strains.

The results of this investigation confirm those of Vidor and Miller (9), with *R. japonicum*, that biotic factors are important in controlling rhizobial populations in moist soil exposed to moderate temperatures. Some of the biotic factors that may be responsible for the death of rhizobia in the nonsterilized flooded soil are bacteriophage (9), *Bdellovibrio* (4), and protozoa (7). The population of *Rhizobium trifolii* and *R. japonicum* in sterile and nonsterile soil was not significantly different when soil moisture content was approximately 50% of field capacity, but the decline in population was substantial in nonsterile saturated soil (7). Moreover, the population of indigenous protozoa did not increase appreciably in moist soil, but did increase appreciably in saturated soil and coincided with a rapid decline in the number of rhizobia (7). Survival of cowpea rhizobia in our study was very good in moist nonsterilized soil, which indicates that the population decline in saturated nonsterilized soil was more likely due to protozoa than to bacteriophage or *Bdellovibrio*. If this soil contained bacteriophage or *Bdellovibrio* the rhizobial populations in nonsterile moist soil would also have been expected to be reduced (7).

Strains of rhizobia are commonly selected for inoculant production based solely on their sym-

biotic capacity to fix nitrogen. Date (2) emphasized the need for inoculum strains to have saprophytic competence. Our results clearly demonstrate that strains of cowpea rhizobia have different capacities for survival in soil exposed to moisture and temperature conditions that commonly occur both in tropical and temperate regions. Temperature and moisture interacted in influencing survival of strains, which indicates that rhizobia should be tested for survival at more than one temperature and moisture content. Because survival of rhizobia in nonsterilized soils was different than in sterilized soils, it appears that survival tests should be conducted with nonsterilized soils even though enumeration of rhizobia under such conditions is more difficult. Data on survival of rhizobia in soil should be considered with information on nitrogen-fixing capacity when recommending new rhizobial strains for inoculants.

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